

phosphorothioate linkages. Unreacted functionalities were capped with a 50:50 mixture of tetrahydofuran/acetic anhydride and tetrahydrofuran/pyridine/1-methyl imidazole. Trityl yields were followed by the trityl monitor during the duration of the synthesis. The final DMT group was left intact. The oligonucleotides were deprotected in 1 mL 28.0-30% ammonium hydroxide (NH_4OH) for approximately 16 hours at 55 °C. Oligonucleotides were also made on a larger scale (20 $\mu\text{mol}/\text{synthesis}$). Trityl groups were removed with just over 8 mL of trichloroacetic acid. All standard amidites (0.1M) were coupled twice per cycle (13 minute coupling step). All novel amidites were also coupled four times per cycle but the coupling time was increased to approximately 20 minutes (delivering 480 μL of amidite). Oxidation times remained the same but the delivery of oxidizing agent increased to approximately 1.88 mL per cycle. Oligonucleotides were cleaved and deprotected in 5 mL 28.0-30% NH_4OH at 55 °C, for approximately 16 hours.

Table I

3'-*O*-(2-methoxyethyl) containing 2'-5' linked oligonucleotides.

SEQ ID (ISIS) #	Sequence (5'-3')¹	Backbone	Chemistry
NO. #			
4 (17176)	ATG-CAT-TCT-GCC-CCC-AAG-GA*	P=S	3'- <i>O</i> -MOE
5 (17177)	ATG-CAT-TCT-GCC-CCC-AAG-G*A*	P=S	3'- <i>O</i> -MOE
6 (17178)	ATG-CAT-TCT-GCC-CCC-AAG _o -G _o A* P=S/P=O		3'- <i>O</i> -MOE
7 (17179)	A*TG-CAT-TCT-GCC-CCC-AAG-GA*	P=S	3'- <i>O</i> -MOE
8 (17180)	A*TG-CAT-TCT-GCC-CCC-AAG-G*A*	P=S	3'- <i>O</i> -MOE
9 (17181)	A _o TG-CAT-TCT-GCC-AAA-AAG _o -G _o A*	P=S/P=O	3'- <i>O</i> -MOE

ISIS-4789

PATENT

10	(21415) A*T*G-CAT-TCT-GCC-AAA-AAG-G*A*	P=S	3'-O-MOE
11	(21416) A* ₀ T* ₀ G-CAT-TCT-GCC-AAA-AAG ₀ -G* ₀ A*	P=S/P=O	3'-O-MOE
	(21945) A*A*A*	P=O	3'-O-MOE
	(21663) A*A*A*A*	P=O	3'-O-MOE
	(20389) A*U*C*G*	P=O	3'-O-MOE
12	(20390) C*G*C*-G*A*A*-T*T*C*-G*C*G*	P=O	3'-O-MOE

¹All nucleosides with an asterisk contain 3'-O-(2-methoxyethyl).

EXAMPLE 50

General Procedure for purification of oligonucleotides

[0187] Following cleavage and deprotection step, the crude oligonucleotides (such as those synthesized in Example 49) were filtered from CPG using Gelman 0.45 µm nylon acrodisc syringe filters. Excess NH₄OH was evaporated away in a Savant AS160 automatic speed vac. The crude yield was measured on a Hewlett Packard 8452A Diode Array Spectrophotometer at 260 nm. Crude samples were then analyzed by mass spectrometry (MS) on a Hewlett Packard electrospray mass spectrometer and by capillary gel electrophoresis (CGE) on a Beckmann P/ACE system 5000. Trityl-on oligonucleotides were purified by reverse phase preparative high performance liquid chromatography (HPLC). HPLC conditions were as follows: Waters 600E with 991 detector; Waters Delta Pak C4 column (7.8X300mm); Solvent A: 50 mM

triethylammonium acetate (TEA-Ac), pH 7.0; B: 100% acetonitrile; 2.5 mL/min flow rate; Gradient: 5% B for first five minutes with linear increase in B to 60% during the next 55 minutes. Larger oligo yields from the larger 20 μ mol syntheses were purified on larger HPLC columns (Waters Bondapak HC18HA) and the flow rate was increased to 5.0 mL/min. Appropriate fractions were collected and solvent was dried down in speed vac. Oligonucleotides were detritylated in 80% acetic acid for approximately 45 minutes and lyophilized again. Free trityl and excess salt were removed by passing detritylated oligonucleotides through Sephadex G-25 (size exclusion chromatography) and collecting appropriate samples through a Pharmacia fraction collector. Solvent again evaporated away in speed vac. Purified oligonucleotides were then analyzed for purity by CGE, HPLC (flow rate: 1.5 mL/min; Waters Delta Pak C4 column, 3.9X300mm), and MS. The final yield was determined by spectrophotometer at 260 nm.